On the Physiology and Pathophysiology of Antimicrobial Peptides

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Antimicrobial peptides (AMP) are a heterogeneous group of molecules involved in the nonspecific immune responses of a variety of organisms ranging from prokaryotes to mammals, including humans. AMP have various physical and biological properties, yet the most common feature is their antimicrobial effect. The majority of AMP disrupt the integrity of microbial cells by 1 of 3 known mechanisms—the barrel-stave pore model, the thoroidal pore model, or the carpet model. Results of growing numbers of descriptive and experimental studies show that altered expression of AMP in various tissues is important in the pathogenesis of several gastrointestinal, respiratory, and other diseases. We discuss novel approaches and strategies to further improve the promising future of therapeutic applications of AMP. The spread of antibiotic resistance increases the importance of developing a clinical role for AMP.

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INTRODUCTION

Cancer and cardiovascular diseases are considered to be the major health problems of the developed world. In contrast, infectious diseases are still the most common cause of death in developing countries. Although effective treatments are available to conquer most infections, during the past decades the misuse of antibiotics has led to horizontal gene transfer among microbes and stimulated their evolutionary potential to develop resistance against conventional antimicrobials. New agents and new therapeutic approaches are needed that will at least temporarily overcome the resistance problem. Because they are products of long-term evolution, antimicrobial peptides (AMP) may offer such a solution. Current molecular biotechnology enables large-scale production of AMP and their use in various applications. Increased effectiveness and specificity of AMP can be achieved by using *in vitro* evolution. More studies focusing on AMP are needed, not only because of their commercial and biotechnological applications but also (and even more importantly) because of the lack of research on bringing AMP from the bench to the bedside. In this review we provide basic information about the physiology of AMP, presenting selected pathophysiological aspects as well as potential applications.

PHYSIOLOGY OF AMP

AMP are a component of the basic defense line of innate immunity (1,2). Peptides with antimicrobial activity were first described by Zeya and Spitznagel in 1966 (3) and named defensins because of their function in host defense (4). Since then, many other peptides with similar antimicrobial effects have been discovered and characterized by use of genetic and molecular biological research methods (5). More recently, investigations have been conducted with bioinformatic approaches such as the basic local alignment search tool (BLAST) and computer simulations (6,7).

AMP act as endogenous antibiotics by direct destruction of microorganisms. Owing to their diverse roles, they are also known as multifunctional peptides. AMP, polypeptides containing fewer than 100 amino acid residues (8), have broad activity spectra that are unique for each peptide. Several AMP are able to simultaneously attack various microorganisms, including Gram-positive and Gram-negative bacteria, fungi, parasites, enveloped viruses, and even tumor cells (9). The antibiotic spectra of AMP are determined by their amino acid sequence and structural conformation (10). Organisms producing AMP include virtually all higher eukaryotes—including plants and invertebrates (11), and also eubacteria and archea (12,13). In humans, several cell types synthesize and secrete AMP—epithelial and professional host-defense cells such as neutrophils, macrophages, and natural killer cells.

The classification of AMP is difficult owing to their considerable diversity. On

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the basis of structural homology motifs, two main families of eukaryotic AMP can be described: cationic antimicrobial peptides and noncationic antimicrobial peptides (14). Cationic peptides, the largest group of AMP, include defensins and cathelicidins. Defensins are open-ended 4–5-kDa peptides with six (or eight in some insect and plant defensins) conserved disulfide-linked cystein motifs. The four defensin families differ in the spatial distribution of cystein residues and in the connectivity of their cystein residues (Figure 1) (8,15). The other classes of cationic peptides are the amino acid enriched class (including histatins), cecropins/magainins, and peptides related to histones or lactoferrin.

The family of noncationic AMP is smaller than the family of cationic peptides, and their antimicrobial activity is considerably lower. There are three subfamilies of noncationic AMP (14): neuropeptide-derived molecules from infectious exudates of cattle and humans (16); aspartic acid–rich molecules, with one member, dermcidin, found in human blood and urine (17,18); and peptides derived from oxygen-binding proteins of arthropods or vertebrates (19,20).

Bacterial strains can also produce AMP to improve their survival and competitive advantages in their microecological niche. The most relevant AMP from bacteria are bacteriocins. These 1.9–5.8-kDa peptides are produced by Gram-positive bacteria. Cationic, anionic, and neutral bacteriocins are targeted against closely related organisms sharing the same niche, and evidence also exists indicating activity against a wide range of human pathogens (21). The most common bacteriocins, lantibiotics, are produced by lactic acid bacteria. Some bacteriocins contain unusual amino acids with post-translational modifications (22); lantibiotics contain the unusual amino acid lanthionine. Bacteriocins can be encoded on plasmids (23,24) and thus spread easily via horizontal gene transfer. This fact is relevant for the use of bacteria with antimicrobial activity for human therapy or for applications in food safety (25).

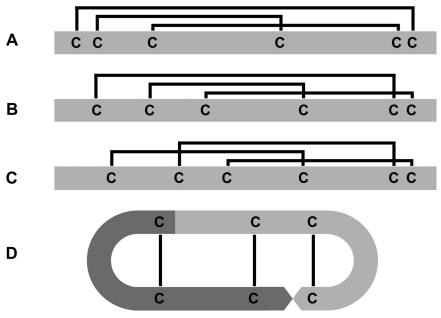

Figure 1. Organization of disulfide bridges between cystein residues in defensin groups: (A) disulfide linkages in α-defensins (1–6, 2–4, 3–5), (B) disulfide linkages in β-defensins (1–5, 2–4, 3–6), (C) disulfide linkages in insect defensins (1–4, 2–5, 3–6), (D) disulfide linkages and structure of θ-defensins.

In addition to standard AMP, other proteins with antimicrobial effects are known. Lysozyme was the first protein reported to have antimicrobial activity (26). Later, the antimicrobial activity of histones was demonstrated (27). Since then many other antimicrobial proteins have been described, including granulysin, produced by natural killer cells and CD8 T cells (28); calprotectin (29); bactericidal/permeability-increasing protein from human neutrophils (30); human lactoferrin (31); and histidine-rich glycoprotein (32).

AMP MECHANISMS OF ACTION

The precise mechanism of action is currently not completely known for all AMP. Several theories have been proposed to explain the molecular processes induced by AMP, but it is currently unknown which of the hypothesized mechanisms is closest to reality. Several models that particularly address the actions of defensins and linear amphipatic cationic peptides propose formation of channels through and/or disruption of bacterial membranes (33,34).

Pore Formation

Killing of bacteria via pore formation in the bacteria membrane requires three principal steps: binding to the bacterial membrane, aggregation within the membrane, and formation of channels. The channel formation leads to leakage of internal cell contents and cell death. An AMP must cross the negatively charged outer wall of Gram-negative bacteria, which contains lipopolysaccharides (LPS), or the outer cell wall of Gram-positive bacteria, which contains acidic polysaccharides (35). In many cases the metabolic activity of target microbes is a critical condition for pore formation (36). The three well-established models for pore formation are the barrel-stave pore (37), the thoroidal pore (38), and the carpet model (39).

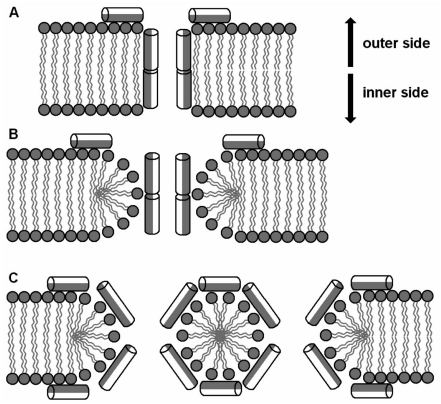

Figure 2. Mechanism of action of antimicrobial peptides—channel formation. The cylinders represent antimicrobial peptides (hydrophobic areas are gray; and hydrophilic, white). (A) Barrel-stave pore model; (B) thoroidal pore model; (C) carpet model.

Barrel-Stave Pore Model

In the barrel-stave pore model, AMP form dimers or multimers after binding to the negatively charged bacterial membrane. The peptide assembly is a crucial step for pore formation (40). Multimers of AMP cross the cell membrane so that the hydrophobic part is facing the lipid bilayer and the hydrophilic part is facing the lumen of the pore. The assembled peptides form barrel-like channels resembling staves (Figure 2A) (41).

Thoroidal Pore Model

The mechanisms of the thoroidal pore model share common features with a

barrel-stave pore formation model, but the AMP form a monolayer by connecting the outer and inner lipid layers in the pore (Figure 2B) (38).

Carpet Model

In this model peptides first cover the outer surface of the membrane like a carpet and then act like detergents, disrupting the membrane bilayer after reaching a threshold concentration. The threshold concentration can be reached after the entire surface is covered with AMP or after local peptide assembly and carpet formation (42). The pores are formed from micelle-like units (Figure 2C).

Molecular Electroporation

Some peptides are able to create electrostatic potential across the bilayer sufficient for pore generation by electroporation. For pore formation, a sufficient charge density must be reached, represented by high contents of cationic amino acids in AMP (33,43).

Sinking-Raft Model

Amphipathic peptides can cause imbalance by binding and sinking into the structure of the lipid bilayer. These peptides may create transient pores lethal for microbes (34,44).

Alternative Mechanisms of Action

Most AMP kill bacteria by pore formation in lipid membranes, but other mechanisms of action have been described and proposed (45). Defensins and cathelicidins can inactivate bacterial LPS by binding to the endotoxin moieties (1). Many peptides act directly inside the microorganisms by inhibiting intracellular processes. The aggregate-channel model (46) features a mechanism of transport through the lipid bilayer without the formation of a stable channel. Some AMP inhibit DNA synthesis (47), protein synthesis (48), or both (49). Histatin targets the mitochondria of fungal pathogens (50). On the other hand, evidence indicates that in addition to pathogen killing, AMP also affect pathogen metabolism. In some cases they can trigger the production of virulence factors, such as the hyaluronic acid capsular polysaccharide (51).

Immunomodulatory Function

AMP bind to cellular receptors and participate in a variety of processes related to the immune response, ranging from inflammation and chemoattraction to wound healing. AMP take part in the chemoattraction of monocytes, T cells, dendritic cells, neutrophils, and mast cells (52,53). AMP themselves are regulated by cytokines produced by immunocompetent cells (54). Some chemokines have antimicrobial properties, a finding that reveals the complexity of these immunomodulatory mechanisms (55). AMP participate in the regulation of the complement system (56), immunoglobulin production, and phagocytosis (57). However, there is accumulating evidence that AMP play an important role in activating the adaptive immune response. Unlike α-defensins, human β-defensins (hBD) were found to be upregulated in respiratory papillomatosis, indicating that hBD might contribute to innate and adaptive immune responses targeted against papillomavirus-induced epithelial lesions (58). Moreover, AMP take part in the interconnection between innate and adaptive immunity (59,60). More detailed overviews on the immunomodulatory functions of AMP are provided elsewhere (54,59).

AMP RESISTANCE

Pathogens are exposed to AMP in many organisms and tissues. The development from previously-sensitive strains to strains resistant to these natural antibiotics is difficult, if not impossible (61). Naturally occurring resistance is also extremely rare. The relative resistance of some human pathogens to these host defense molecules is now accepted as an important factor of virulence. Bacteria with AMP resistance also exhibit a much higher resistance to standard antibiotics (62). The increased pathogenicity of AMP-resistant strains and the knowledge of the molecular basis of AMP resistance may provide new targets for antimicrobial therapy of infectious diseases.

There are two mechanisms of resistance: inherent resistance (constitutive resistance) and adaptive resistance (inducible resistance) (63). In inherently resistant strains the factors ensuring resistance are always present. Several mechanisms enable inherent resistance: lack of electrostatic affinity for AMP, altered membrane energetics, and electrostatic shielding. AMP must get through various enveloping structures such as LPS in the outer membranes of Gram-negative bacteria, or thick cell walls of Gram-positive bacteria coupled with cross-linked peptidoglycans and teichoic or lipoteichoic acids (64). Some microorganisms lack electrostatic affinity to AMP. In some resistant *Staphylococcus* species unique lipid and phospholipid composition has been described (65). Resistant *Staphylococcus aureus* strains have high D-alanylation of teichoic aids with positively charged amine groups, which lower the negative charge of the cell wall (55).

Microorganisms with altered membrane energetics, such as a respiration-deficient mutant of *Candida albicans* (66) with lowered mitochondrial ATP synthesis or *S. aureus* strains with constitutively reduced transmembrane potential (67), are more resistant to AMP than those with normal energy status. Electrostatic shielding of microorganisms can be explained by the presence of highly anionic glycocalyx or a special capsule with the ability to shield the lipid bilayer from AMP.

Inducible resistance is based on activation of factors needed for survival in the presence of sublethal levels of AMP. In many cases two-component regulatory systems are included in this mechanism, such as *PhoP/PhoQ* in Gram-negative pathogens. Inducible resistance involves mostly extracellular structural modifications, protease-mediated resistance, efflux-dependent mechanisms, and modification of intracellular targets (63). AMP can function as ligands for the bacterial sensory kinase PhoQ for the initiation of virulence and adaptive responses. Thus, there are concerns that therapeutic administration of AMP could exacerbate infections by

promoting bacterial virulence and select resistant mutants by giving advantage to adaptive behavior (68). On the other hand, understanding of inducible resistance provides a rational basis for the optimization and selection of suitable AMP. Recently, determination of the genome-wide gene regulatory response to human hBD-3 in nosocomial pathogen *Staphylococcus epidermidis* revealed that Gram-positive bacteria have developed an efficient and unique three-component AMP-sensing system that controls resistance mechanisms to AMP (69). Components of this system are promising targets for antimicrobial drug development. Indices also exist that indicate the limited host range of some bacterial pathogens may be at least partially caused by differences in bacterial susceptibility to host AMP (70).

AMP IN ACTION

Disease-causing microbes that have become resistant to conventional antibiotics are an increasing public health problem. There is evidence that about 70% of bacteria-causing infections in hospitals are resistant to at least one of the commonly used antibiotics (71). There are also multiresistant microorganisms, some of which are resistant to nearly all approved antibiotics (72).

AMP, with their diversity in structure and chemical nature, are a new alternative to conventional antibiotics. The probability of the development of pathogen resistance and/or side effects is much lower with AMP than chemical antibiotics, because AMP are naturally a part of human antimicrobial defense. Therefore, AMP are considered to be the basic element of novel antibacterial, antifungal, and antiviral drugs in the therapy of infectious diseases (73–75) and parasitic infections (76), and AMP may also be useful in the treatment of cancer (9,77,78) and HIV infection (79).

Induction of AMP Expression

Proinflammatory cytokines, certain bacterial strains as well as other exogenous compounds, have been identified as inducers of endogenous AMP expression (54,80,81). Schlee and colleagues investigated the stimulatory effect of probiotic bacterial strain *Escherichia coli* Nissle 1917 on hBD-2 expression and identified the bacterial factor responsible for hBD-2 induction (82). These investigators found that the stimulatory effect of this bacterial strain on hBD-2 expression *in vitro* is dominantly mediated through the presence of flagellin. Addressing the not-fully-explained link between psychological stress and increased susceptibility to microbial infections, Aberg *et al*. showed that psychological stress decreases the levels of two key AMP in the skin via increased endogenous glucocorticoid production (83). These data suggest that glucocorticoid blockade could normalize cutaneous antimicrobial defense during psychological stress. Recently, Rabiq *et al*. provided an alternative to conventional treatment of acute infectious diseases such as *Shigella* infections (84). Based on results of animal experiments, Rabiq *et al*. suggest that orally administered sodium butyrate can mediate a therapeutic effect via induction of endogenous AMP expression and secretion in the colon and rectum. Similar findings were observed in a study investigating the effect of the hormonally active form of vitamin D_3 ($1,25(OH)_2D_3$) on expression of cathelicidin in both normal and cystic fibrosis bronchial epithelial cell lines (85). Vitamin D stimulated the expression and secretion of endogenous cathelicidin, inducing antimicrobial activity against airway pathogens *Bordetella bronchiseptica* and *Pseudomonas aeruginosa*. Before human trials begin, however, many unsolved questions should be answered to fully elucidate the mode of action of exogenously administered agents (such as butyrate or vitamin D) in inducing innate immunity mechanisms.

Inflammation

AMP are key components of innate host defense on various sites of the body. Results of a study by Beisswenger *et al*. show that an allergic airway inflammation suppresses the innate antimicrobial host defense (86). Another study provides evidence that human AMP (hBD, cathelicidin LL-37) participate in cutaneous inflammation and wound healing by inducing keratinocyte migration and proliferation and production of proinflammatory cytokines/chemokines (87). Using *in vivo* studies on contact dermatitis and *in vitro* studies of dendritic cell function, Di Nardo and colleagues present an immunosuppressive role of cethelicidin and try to explain the mechanism for this effect by describing a novel membrane-dependent mechanism (88). Cathelicidin LL-37 also causes functional changes in mast cells (increased expression of TLR4 and release of interleukin [IL]-4, IL-5, and IL-1β), leading to direction toward innate immunity (89).

Infections

Cathelicidins kill bacteria rapidly through permeabilization of bacterial cell membranes and binding to LPS. These features enabled different cathelicidins to be effective in decreasing lethality in rat models of septic shock after intravenous application (90), and in reducing mortality of staphylococcal sepsis in mice after parenteral application (91). Cathelicidin LL37 and its ortholog CRAMP seemed to be key defense factors in a mouse model of urinary *E. coli* infection and in human mucosal immunity of the urinary tract, respectively (92). The role of LL-37 in pathogenesis of various clinical entities has been investigated in many studies. Cirioni *et al*. demonstrated that LL-37 effectively protects rats against lethal sepsis caused by Gram-negative bacteria, suggesting a future role in treatment of sepsis (93). Other data indicate that enhanced cathelicidin-related innate immunity has protective effects in sepsis (94). Cathelicidin has been further shown to promote gastric ulcer healing in rats by enhancing cell proliferation and angiogenesis (95). MBI-226, the synthetic cationic peptide, can be used for the treatment and prevention of various infections. In 2000, a phase III trial of MBI-226 for the prevention of catheter-related bloodstream infections was initiated (96). Another interesting finding is the cardioprotective effect of proline/arginine-rich

PR-39 in myocardial ischemia–reperfusion (97,98). The antimicrobial activity of the amphibian-derived K_4-S4(1-15)a against oral pathogens associated with caries and periodontitis was tested *in vitro*. Results show that compared with resistance to human AMP (LL-37), K_4-S4(1-15)a demonstrated the highest activity against *Streptococcus mutans*, *Streptococcus sobrinus*, *Lactobacillus paracasei*, and *Actinomyces viscosus*. This effect was also profound in surface-attached and biofilm-grown *S. mutans*, suggesting novel AMP may play a role in prevention and treatment of oral diseases (99). AMP are important subjects in the process of host defense against inhaled pathogens. LL37, a multifunctional human cathelicidin, plays a role in lung infection and inflammation (100). Furthermore, the human histatin 5 derivative P-113 showed potent activity against important respiratory pathogens such as *P. aeruginosa*, *S. aureus*, and *Haemophilus influenzae*. It has been shown that P-113 reduces plaque, gingivitis, and gingival bleeding in a human experimental gingivitis model, and phase I and II clinical trials indicated no side effects (101). Protegrin-1, a member of the θ-defensin–like family, can be an effective antimicrobial agent in cystic fibrosis lung infections (102). In a phase I trial, the aerosol form of the synthetic protegrin isegan was demonstrated to be effective in the treatment of respiratory infections in cystic fibrosis patients and the gel form in the treatment of pneumonia (103). Amelioration of oral mucositis by reducing microflora densities on the mucosal surfaces of the mouth was shown in a hamster model (104). In cystic fibrosis patients the high content of salt in mucus inhibits salt-sensitive AMP, one of the reasons for frequent airway infection in these patients (105). This inhibition can be bypassed by the administration of salt-insensitive derivates of AMP into airways.

Noninfectious Diseases

The nonantimicrobial effects of AMP also include mediation of immune-cell–induced death of vascular smooth muscle cells in atherosclerosis. A study by Ciornei *et al*. showed that LL-37 is present in atherosclerotic lesions and that it induces death of vascular smooth muscle cells via development of apoptosis triggered by an initial mild perturbation of plasma membrane integrity (106). Another study also demonstrated the increased expression of LL-37 in atherosclerotic lesions, mainly in macrophages but also in endothelial cells or T cells, indicating its role in enhancing the innate immunity in atherosclerosis (107). Interestingly, nuclear localization of hBD-1 was demonstrated, suggesting a role for AMP in gene expression and providing new data shedding light on mechanisms of defensin functions (108). Results also showed hBD-1 sequence homology with cationic nuclear localization signal sequences, making the effect of AMP more complex than previously thought.

In addition, AMP has been reported to have antitumor activity (109). This activity is possible because of differences in membrane composition of transformed cells. These differences (for example, higher phosphatidylserine content) can result in higher sensitivity to membrane-permeabilizing peptides. Magainins lyse many types of tumor cells at five- to ten-fold lower concentrations than toxic concentrations for nonmalignant cells (110,111). The magainin-related cecropins also have antitumor activity in human cells (112). In one of the original studies, human and rabbit defensins reduced the oncogenicity of murine teratocarcinoma cells *in vivo* (113). Moreover, synthetic antitumor peptides derived from frog antimicrobial peptides have been proven to yield positive outcomes as anticancer agents (114). There is also promising evidence that AMP from the Cecropin family have potent antitumor activity proven via inhibition of proliferation and viability of bladder cancer cells (9). Moreover, findings of Gambichler and colleagues showing altered expression patterns of hBD-1 and hBD-2 indicate that hBD may also play a role in pathogenesis of basal cell carcinoma (78). On the other hand, there are many reports of mitogenic activity of AMP (60), showing that this field needs further studies.

Probiotics

There is evidence that hBD-2 inhibits the growth of *Helicobacter pylori in vitro*, suggesting that hBD-2 plays a role in *H. pylori*–induced gastritis (115). The prokaryotic antimicrobial peptide nisin is another possible treatment for *H. pylori*–caused gastric ulcers (116). Nisin (generally recognized as safe) together with other bacteriocins has proven effective in animal production, and its protective effects against some bacterial infections (*Salmonella typhimurium*, *Salmonella pullorum*, and *Listeria innocua*) have been examined. The administration of bacteriocin-producing bacteria in animal production is considered to be more cost-effective than direct peptide administration (117).

Probiotics producing AMP can be effective antiinfection and antiinflammation agents in the human gastrointestinal tract. *Lactobacillus ruminus* SPM 0211, a probiotic microbe, completely inhibited the growth of vancomycin intermediate-resistant *S. aureus* and vancomycin-resistant Enterococci after 9 hours of incubation (118). Antimicrobial and immunomodulatory activities of *Bacillus clausii* probiotic strains have been evaluated *in vitro* on Swiss and C57 Bl/6j murine cells (119). Furthermore, one *Bacillus pumilus* strain and one *Bacillus cereus* strain were found to exhibit a bacteriocin-like activity against other *Bacillus* species (120). These and many other findings suggest that beneficial activities of probiotic strains are also achieved through their AMP production. Techniques of genetic engineering could further increase the AMP production of these bacteria, and suitable production strains could be created for alternative gene therapy applications (121). Such recombinant probiotics might be useful for Crohn disease or ulcerative colitis treatment, because some evidence indicates that the lack of mucosal peptide antibiotics may play a

pivotal role in the etiopathogenesis of these diseases (122,123). In a gene therapy study, local injection of a plasmid carrying rat cathelicidin gene promoted gastric ulcer healing in rats (95). These findings can be partially explained through mutations in nucleotide-binding oligomerization domain protein 2 (NOD2), which lead to a predisposition to Crohn disease. These NOD receptors are sensors of mucosal bacterial community and may regulate gut antimicrobial peptide expression (124). Local deficiency of AMP may represent a biological factor that contributes to development of various other pathological entities including dental caries (125) and bacterial vaginosis (126).

Gene Therapy

An interesting approach has been described in which gene therapy methods were used to deliver the *LL-37* gene into cystic fibrosis xenografts (127). In another gene therapy study, adenovirus-mediated gene transfer of the antimicrobial peptide elafin increased the antimicrobial activity of mouse lung cells against *S. aureus in vitro* and *in vivo* (128). In exploring potential treatment approaches, the use of bacterial gene therapy strategies (alternative gene therapy and bactofection) is interesting, because of higher selectivity of bacteria for airway cells compared with other gene therapy vectors (129). In general, application of antimicrobial peptides by using gene therapy can be more effective than direct peptide use. In a recent gene therapy study, the cutaneous adenoviral delivery of human cathelicidin was significantly more effective than the administration of synthetic host defense peptides in the treatment of burn wound infections (130).

CONCLUSION

In conclusion, according to the results of experimental and clinical studies, AMP play a role in various physiological processes, mostly in innate immunity. These processes, however, must be investigated in detail to assess the exact function of all relevant AMP and to uncover the extent to which AMP influence the etiopathogenesis of candidate diseases, such as Crohn disease. Knowledge of physical and chemical properties of AMP underlies the complete understanding of their mechanisms of action. We have summarized current knowledge with emphasis on advances in biomedical use.

Even though AMP have been known for decades, they still provide research challenges and are prospective agents in the fight against infections and other major diseases, mainly because they are gene encoded and occur naturally in the human body. Advanced expression systems enable large-scale production of therapeutically relevant AMP, which can be potentially used in the treatment of microbial infections. To better understand the nature of AMP it is necessary to assess the functional consequences of genetic polymorphisms and mutations in genes encoding human AMP. These data will allow elucidation of correlations between impaired AMP expression and diseases.

Beyond direct application of specific AMP as proteins, genes encoding AMP can also be delivered as gene therapy. The most promising treatment under investigation in this area is alternative gene therapy using genetically-modified bacteria producing therapeutic AMP *in situ* for targeted killing of specific pathogenic species, a treatment that can be especially suitable in the treatment of dental caries, Crohn disease, and other disorders in which disturbances in natural microflora play a role and host-microbe balance must be preserved. Currently, in the era of antibiotic resistance, AMP is a desired novel tool with proven efficiency and the potential for long-term application.

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DISCLOSURE

We declare that the authors have no competing interests as defined by *Molecular Medicine*, or other interests that might be perceived to influence the results and discussion reported in this paper.

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